



A CASE STUDY

Role and its utilization of beneficial micro-organisms for sustainable crop production

S.K. CHOUDHARY*, S.K. GUPTA, M.K. SINGH AND S. SHERAZ MAHDI

Department of Agronomy, BAC, Bihar Agricultural University, Sabour, BHAGALPUR (BIHAR) INDIA

(Email : saurabhkkv2885@gmail.com)

Abstract : Soil micro-organisms are important component of integrated nutrient management and soil biodiversity system. They play a pivotal role in the functioning of plants by influencing their physiology and development. It is very important role in biogeo-chemical cycles and has been used for crop production for decades. Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Soil bacteria which are beneficial to plant growth, referred to plant growth promoting rhizobacteria (PGPR), which are capable of promoting plant growth by colonizing the plant root. Symbiotic nitrogen-fixing bacteria include the *Cyanobacteria* of the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium*. Plant growth promotion and development can be facilitated both directly and indirectly. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms. This can be achieved by the production of siderophores, i.e. small metal-binding molecules. Biological control of soil-borne plant pathogens and the synthesis of antibiotics have also been reported in several bacterial species. Another mechanism by which PGPR can inhibit phytopathogens is the production of hydrogencyanide (HCN) and/or fungal cell wall degrading enzymes, e.g., chitinase and β -1,3-glucanase. Direct plant growth promotion includes symbiotic and non-symbiotic PGPR which function through production of plant hormones such as auxins, cytokinins, gibberellins, ethylene and abscisic acid. Production of indole-3-ethanol or indole-3-acetic acid(IAA), PGPR also help in solubilisation of mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates, and improve soil structure and organic matter content. PGPR retain more soil organic N, and other nutrients in the plant–soil system, thus, reducing the need for fertilizer.

Key Words : PGPR Symbiotic, Non-symbiotic, P and K solubilisation, Phytohormones, Bio control

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INTRODUCTION

Soil micro-organisms are very important component of integrated nutrient management and soil biodiversity system. They play a pivotal role in the functioning of plants by influencing their physiology and development. In recent years, there is use of chemical fertilizer and

pesticide use more than critical limit which not only deteriorate soil health but also create several environmental impacts as global threat. Hence, introduction of beneficial micro-organisms in agricultural for sustainable agriculture is very important. The micro-organism is better compared to the chemical fertilizers like a) they are more safe, b) show reduced environmental

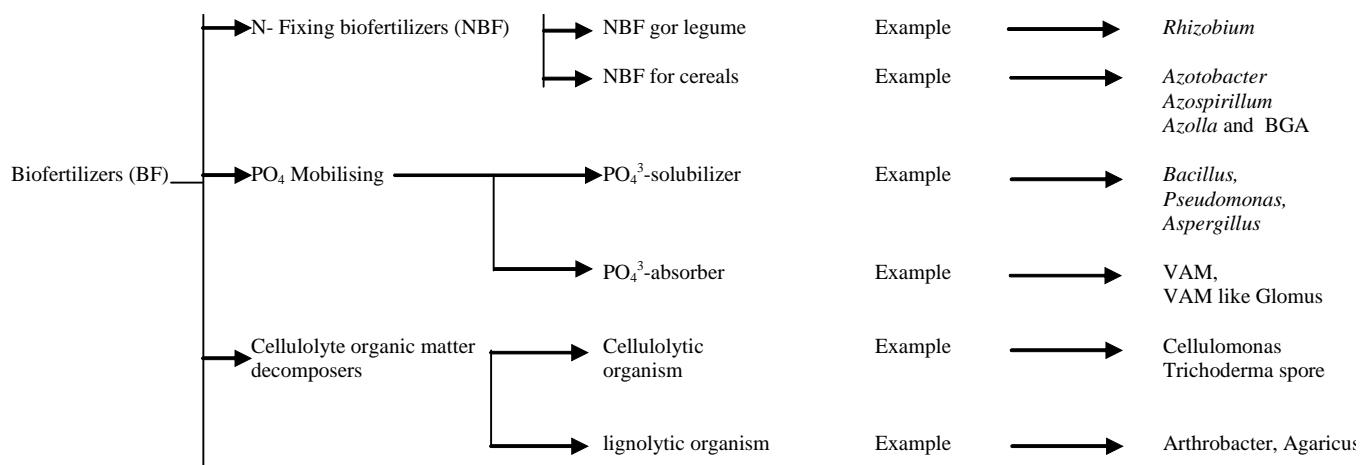
* Author for correspondence

damage, c) show more targeted activity, d) are effective in smaller quantities, e) are able to multiply but at the same time controlled by the plant and indigenous microbes, f) have quicker decomposition procedures, g) are less likely to induce resistance by the pathogens and pests and finally g) can be used either in organic and conventional agriculture etc. For example plant growth promoting *rhizobacteria* (PGPR) are free living bacteria of beneficial agricultural importance. The PGPR present encourage beneficial effects on plant health and growth, suppress disease causing microbes and accelerate nutrient availability and assimilation. Thus, in the quest to improve soil fertility and crop yield and to reduce the negative impacts of chemical fertilizers on the environment, PGPRs can induce plant's growth either directly or indirectly. Direct mechanisms comprise the production of substances like phytohormones, liberation of nutrients and stimulation of induced systemic resistance. For example, diazotrophs, phosphate (P) solubilizing bacteria (PSB) viz., Rhizobia group, *Azospirillum*, *Agrobacterium*, *Pseudomonas* and *Dyadobacter*, etc. On the other hand, indirect mechanisms comprise stimulation of symbiotic relationships, root growth stimulation and biocontrol. For example, genera like *Azospirillum*, *Bacillus* and *Pseudomonas* can enhance legume symbioses. Moreover, it is important to know that in some cases, several mechanisms are involved when it comes to beneficial plant microbe interactions. Thus, the identification of the mechanisms responsible of plant growth represents a big challenge in current scenario. PGPR exist in the rhizosphere These bacteria belong to the genera *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azoarcus*,

Azospirillum, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Dexia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoae*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas* and *Zoogloea*. Some PGPR may have more than one mechanism for accomplishing plant growth (Ahmad *et al.*, 2008). Included in such mechanisms are antagonism to pathogenic fungi, siderophore production, nitrogen fixation, phosphate solubilization, the production of organic acids and indole acetic acid (IAA), NH₃, HCN, the release of enzymes (soil dehydrogenase, phosphatase, nitrogenase, etc.), and the induction of systemic disease resistance (ISR) (Hayat *et al.*, 2010).

Role of biofertilizers in crop production :

Soil micro-organisms play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant macro and micro nutrients. It is well-recognized that microbial inoculants constitute an important component of integrated nutrient management for sustainable agriculture. Biofertilizer is defined as a preparation of live or latent cells of efficient strains (*Rhizobium*, *Azotobacter*, *P.S. Bacteria*) in sufficient amount used with the objective of increase microbial population and accelerate those microbial process which augment the availability of nutrient that can easily available to crop plant. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes. Biofertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. But it helps to increase the nutrient efficiency.



Advantage of biofertilizers :

- Environment friendly
- Increase nitrogen fixation and nutrient availability
- Cheap and economical
- Non toxic and non-polluting
- Sustain crop productivity
- No residual effect
- Improve soil health
- Decrease use of chemicals
- Multifaceted action
- Socially acceptable

Functions of harmful micro-organisms :

- Induction of plant diseases
- Stimulation of soil-borne pathogens
- Immobilization of plant nutrients
- Inhibition of seed germination
- Inhibition of plant growth and development
- Production of phytotoxic substances

Main micro-organisms used as biofertilizers and their functions Rhizobium :

Rhizobium is leguminous symbiotic N₂-fixing bacteria (bacteriod) inside nodule and it is present in soil and help in formation of root nodule (in root) by infecting the cell of root of leguminous plant. After infection by *Rhizobium* in cells of root of leguminous plant the *Rhizobium* secrete some chemical which stimulate the cells of root to proliferate and make the root nodule inside this nodule the *Rhizobium* live and divide and after some time it perform N₂ fixation in root nodule. The plant roots supply essential minerals and newly synthesized substances to the bacteria. Because of their N-fixing ability, legumes are less reliant on inorganic N fertilizer than many other non-legume crops such as cereals and pasture grasses. N fixation by legumes can also maintain soil fertility and can be of benefit to the following crop. *Rhizobium* inoculation is a well-known agronomic practice to ensure adequate N supply for legumes in place of N fertilizer. It is reported that *Rhizobium* can fix 50-300 kg N/ha.

Azotobacters and Azospirillum :

These are free-living bacteria that fix atmospheric nitrogen in cereal crops without any symbiosis and they do not need a specific host plant. *Azotobacters* are abundant in well-drained, neutral soil. They can fix 15-20 kg/ha N per year. *Azotobacter* sp. can also produce

antifungal compounds to fight against many plant pathogens. They also increase germination and vigour in young plants leading to improved crop stands.

Organic and inorganic phosphate solubilisation bacteria (PSB) :

Phosphorus is an essential nutrient for plants. There are a number of micro-organisms which can solubilize the economical sources of phosphorus, for instance rock phosphate. Among such phosphate solubilizing bacteria are *Pseudomonas striata*, *Enterobacter*, *Erwinia*, *B. megaterium* and *O. anthropi* TRS2 (Chakraborty *et al.*, 2009). They solubilize the bound phosphorus, mineralize organic phosphorus and release soluble inorganic phosphate into soil through decomposition of phosphaterich organic compounds. It is reported that PSB culture increased yield up to 200-500 kg/ha and thus, 30 to 50 kg of super phosphate can be saved.

Potassium solubilizing bacteria (KSB) :

Potassium solubilizing bacteria (KSB) Biofertilizers have been used as source to improve plant nutrients in sustainable agriculture. KSB (*Bacillus mucilaginosus*) it enhance soil availability of potassium solubilizing bacteria (KSB) are able to solublize rock potassium mineral powder, such as mica, illite and orthoclases through production and excretion of organic acids shows that KSB increased K- availability in soil and increased minerals content in plants.

Vesicular arbuscular mycorrhiza (VAM) :

Mycorrhizae are mutually beneficial (symbiotic) relationships between fungi and higher plant roots. VAM fungi infect and spread inside the root. They possess special structures known as vesicles and arbuscules. The plant roots transmit substances to the fungi and the fungi aid in transmitting nutrients and water to the plant roots. The fungal hyphae may extend the root lengths 100-fold. The hyphae reach into additional and wetter soil areas and help plants absorb many nutrients, particularly the less available mineral nutrients such as phosphorus, zinc, molybdenum and copper. Some VAM fungi form a kind of sheath around the root, sometimes giving it a hairy, cottony appearance. Because they provide a protective cover, mycorrhizae increase seedling tolerance to drought, to high temperatures, to infection by disease fungi and even to extreme soil acidity. Application of VAM produces better root systems which combat root

Table 1: Biofertilizers uses in different crops

Sr. No.	Biofertilizers	:	Crops
1.	<i>Rhizobium</i> inoculants	:	Leguminous
2.	<i>Azotobacter</i> inoculants	:	Rice
3.	<i>Azospirillum</i> inoculants	:	Cereals, sorghum, maize, millets, triticum
4.	<i>Cyanobacterial</i> inoculants	:	Rice
5.	<i>Azolla</i>	:	Rice
6.	<i>Phosphobacteria</i> inoculants	:	Crops plants and vegetable
7.	Sea weeds	:	Coconut, barely potato, tomato, citrus
8.	Vesicular arbuscular mycorrhiza (VAM)	:	Maize, rice, cotton, citrus, wheat, tobacco, sugarcane, berries, tea, papaya
9.	Organic matter and composting	:	All crops, vegetable, fruits
10.	Genetically engineered biofertilizers	:	Nitrogen fixation in soil for agriculture crops

Table 2 : Some PGPR and their beneficial effects on plants

Sr. No.	PGPR	Plant	Species	Effect compared with
1.	<i>Serratia marcescens</i> NBRI1213	Betel vine	Decreased the number of diseased plants caused by <i>Phytophthora</i> . Increases in shoot length (81%), shoot dry weight (68%), root length (152%), and root dry weight (290%)	Lavania <i>et al.</i> (2006)
2.	<i>Bacillus subtilis</i> GB03, <i>B.amyloliquefaciens</i> IN937a, <i>B. pumilus</i> SE34, <i>B. pumilus</i> T ₄ , <i>B. pasteurii</i> C9, <i>Paenibacillus polymyxa</i> E681, <i>P.fluorescens</i> 89B61 and <i>S. marcescens</i> 90166	<i>Arabidopsis</i>	Increased foliar fresh weight	Ryu <i>et al.</i> (2005)
3.	<i>P. aeruginosa</i> and <i>Serratia liquefaciens</i>	Broad beans	Better phytoremediation potential of broad bean plants grown in oily sand	Radwan <i>et al.</i> (2005)
4.	<i>Pseudomonas</i> spp.	Wild plants	Increased total microbial activity, shoot and root length, total dry weight	Ahn <i>et al.</i> (2007)
5.	<i>Bradyrhizobium</i> and PGPR	Mung bean	Increased root and shoot growth, nodulation in legume	Shahroona <i>et al.</i> (2006)
6.	<i>Acinetobacter</i> , <i>Alcaligenes faecalis</i> , <i>Bacillus cereus</i> , <i>Enterobacter hormaechei</i> , <i>Pantoae</i> , <i>P.aeruginosa</i>	Wheat	Improved plant growth and nutrition under salt stress	Egamberdieva (2008)
7.	<i>Pseudomonas brassicacearum</i> , <i>P. marginalis</i> , <i>P. oryzihabitans</i> , <i>P. putida</i> , <i>Alcaligene xylosoxidans</i> , <i>B. pumilus</i> and <i>Rhodococcus</i> spp.	Indian Mustard and rape	Increased root elongation incadmium supplemented soil input	Belimov <i>et al.</i> (2005)
8.	<i>P. fluorescens</i> biotype G (ACC5), <i>P. fluorescens</i> (ACC14) and <i>P. putida</i> biotype A (Q7)	Pea	Improved fresh and dry weight, root length, shoot length, number of leaves perplant, andwater use efficiency under drought stress	Zahir <i>et al.</i> (2008)
9.	<i>Pseudomonas</i> BA8, <i>Bacillus</i> OSU142, and <i>Bacillus</i> M3	Strawberry	Increased total soluble solids, total sugar and reduced sugar	Pirlak and Kose (2009)
10.	<i>B. cepacia</i> strain OSU7	Stored potatoes	Biocontrol agent of <i>Fusarium</i> dry rot	Recep <i>et al.</i> (2009)
11.	<i>Agrobacterium rubi</i> strain A 16, <i>Burkholderia gladioli</i> strain BA 7, <i>P. putida</i> strain BA 8, <i>B. subtilis</i> strain BA142, and <i>B. megaterium</i> strain M 3	Radish	Improved the percentage of seed germination under saline conditions	Kaymak <i>et al.</i> (2009)
12.	<i>A. amazonense</i>	Rice	Increased grain dry matter accumulation (7–11.6%), the number of panicles (3–18.6%) and nitrogen accumulation at grain maturation (3.5–18.5%)	Rodrigues <i>et al.</i> (2008)

Source : Olubukola Oluranti Babalola, 2010

rotting and soil-borne pathogens.

Inoculation of biofertilizers :

Biofertilizers are generally applied to soil, seeds or seedlings, with or without some carrier for the micro-organisms, for example, peat, composts or stickers. Regardless of methods, the number of cells reaching the soil from commercial products is smaller than the existing numbers of soil or rhizosphere micro-organisms; these added cells are unlikely to have a beneficial impact on the plant unless multiplication occurs.

Seed inoculation :

Seed inoculation uses a specific strain of microbe that can grow in association with plant roots; soil conditions have to be favourable for the inoculants to perform well. Selected strains of N-fixing *Rhizobium* bacteria have proven to be effective as seed inoculants for legumes. The seed treatment can be done with any of two or more bacteria without antagonistic effect. In the case of seed treatment with *Rhizobium*, *Azotobacter*, *Azospirillum* along with PSB, first the seeds must be coated with *Rhizobium* or *Azotobacter* or *Azospirillum*. When each seed has a layer of the aforesaid bacteria then the PSB inoculant has to be treated on the outer layer of the seeds. This method will provide maximum numbers of population of each bacterium to generate better results. In soil inoculation, microbes are added directly to the soil where they have to compete with microbes already living in the soil that are already adapted to local conditions and greatly outnumber the inoculums.

PGPR bioactive factors :

PGPR bioactive factors are substances that impact on growth. Examples include.

Root exudates :

Root exudates are chemical compounds photosynthates, organic acid, sugar (Castro Sowinski *et al.*, 2007 and Kamilova *et al.*, 2006), polyamine putrescine (Kuiper *et al.*, 2001) excreted from root tissues; The process is called rhizode position. Indirect interactions between plants and microbes occur in the rhizosphere due to root exudates.

Vitamins :

They produce wide range of vitamins (thiamine, biotin, pantothenic acid, folic acid, riboflavin, vitamin B12,

nicotinic acid, pyridoxine and myoinositol), these bacteria are widespread in the rhizosphere. A large proportion of indigenous bacteria requires or is stimulated by water soluble B vitamins and amino acids. They will not grow in simple laboratory media unless supplemented with the appropriate substances. The ability of PGPR to synthesize biotin and/or thiamine appears to be an important colonization trait (Lugtenberg *et al.*, 2001). Thiamine is the most frequently required vitamin, but biotin and vitamin B12 also are essential for a large number of bacteria.

Amino acids :

Most of Biofertilizers are produce different type of amino acids like glutamine and asparagine with glutamic acid, alanine, serine, valine, isoleucine, aspartic and leucine which are also present. The loss of amino acids by exudation has been reported (Alexandre *et al.*, 2000) the amount of loss may vary with plant's species, age and developmental stage. The role of amino acid is to support growth of auxotrophs.

Production of growth promoters phytohormones :

Several bacteria produced Phytohormones like Gibberellins (GAs) which may influence seed germination, stem elongation, flowering, and fruit setting of higher plants. Four GAs (GA_1 , GA_3 , GA_4 and GA_{20}) from seven species of bacteria, *Acetobacter diazotrophicus*, *A. lipoferum*, *A. brasiliense*, *Bacillus licheniformis*, *Bacillus pumilus*, *Herbaspirillum seropedicea* and *Rhizobium phaseoli*, have been identified (MacMillan, 2002). *Azotobacter chroococcum*, an inoculant that fixes atmospheric nitrogen, produces on average 0.05 μ g gibberellic acid (GA_3) and PGPR synthesize auxins and these influence root growth, cell elongation, tissue differentiation, plant growth promotion and responses to light and gravity. Auxins are the most important plant hormone produced by *Azospirillum*, *Bacillus megaterium* and *Pseudomonas* spp. HPLC analysis confirmed the presence of IAA and indoleacetamide (IAM) as the major auxins in the culture filtrates of some rhizobacteria (LopezBucio *et al.*, 2007).

Regulating ethylene production in roots :

Role of ethylene is very known in plants but following seed germination, a sustained high level of ethylene would inhibit root elongation, and lead to abnormal root growth, which would seriously impede plant growth and development. Although ethylene is involved

in the regulation of numerous physiological processes in plants, it is also produced by PGPR (Babalola, 2010). Apart from being a plant growth regulator, ethylene acts as a stress hormone when under conditions such as those generated by salinity, drought, water logging, heavy metals and pathogen city. The bacteria occurring on the root surface degrade 1aminocyclopropane1carboxylate (ACC), ACC to ammonium and α -ketobutyrate for use as carbon and nitrogen sources. The results of (Shaharoona *et al.*, 2006) demonstrated that ACC deaminase activity is an efficient parameter for the selection of promising PGPR under axenic conditions. Ethylene plays a key role in inducing multifarious physiological changes in plants at the molecular level (Saleem *et al.*, 2007).

Production of siderophores :

Low molecular weight siderophores synthesized by PGPR can solubilize and sequester iron from the soil and then provide it to the plant cells. *Ochrobactrum anthropi* TRS2 (Chakraborty *et al.*, 2009) isolated from the rhizosphere of tea produces siderophore and IAA *in vitro*. *Pseudomonas* spp. produces fluorescent siderophores. These bacteria have biological control and growth promoting capability.

Production of volatile compounds :

Low molecular weight metabolites, such as HCN, a biocide, with antifungal activity is released in a blend of volatile organic compounds such as 2,3 butanediol and aceto in that promote the growth of *Arabidopsis thaliana*.

Release of enzymes :

Fixation of N₂ that is transferred to the plant :

Leguminous plants have a symbiotic (mutually advantageous) relationship with the bacteria that provide fixed nitrogen. In the rhizosphere, nitrogen fixing (diazotrophic) bacteria are also present. For Examples *Azotobacter vinelandii*, *Beijerinckia diaerxii* and *Zoogloea* strain Ky1 (Meunchang *et al.*, 2006). Other bacterial genera are *Acetobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Enterobacter*, *Klebsiella* and *Pseudomonas*. These are broadly divided into three categories, viz., symbiotic micro-organism e.g. legume—*Rhizobium* symbiosis, asymbiotic or free living e.g. *Azotobacter* and associative symbiosis, e.g. *Azospirillum*. All are helping in nitrogen fixation in crops.

Production of exoenzymes that suppress deleterious microbes :

PGPR may synthesize some enzymes that modulate plant growth and development. The production of enzymes includes chitinase, cellulase, β 1, 3glucanase, protease, lipase can lyse some fungal cells (Muleta *et al.*, 2007) and suppress deleterious rhizobacteria. Examples of the best characterized defence enzymes are peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (Latha *et al.*, 2009).

Production of antibiotics :

Some *Bacillus* spp. produce antibiotics and promote plant growth (Choudhary and Johri, 2009). The production of phenazine antibiotics contributes to ecological fitness by competing with the resident micro flora. The modes of action of these antibiotics are not well known but they do suppress root diseases. *O. anthropi* TRS2 (Chakraborty *et al.*, 2009) and *Bacillus* sp. MEP2 18 and ARP (2) 3 exhibit antagonistic activity against some phytopathogenic fungi (Principe *et al.*, 2007). Also elaborated are the antifungal properties of pyoluteorin, pyrrolnitrin and iturin.

Nematophagous bacteria :

Nematophagous bacteria exhibit diverse modes of action including promotion of plant health. They act synergistically through the direct suppression of nematodes, promoting plant growth, and facilitating the rhizosphere colonization and activity of microbial antagonists in the rhizosphere.

Actinomycetes :

Actinomycetes (Babalola *et al.*, 2009), when associated to root systems (rhizosphere), are considered rhizobacteria, showing good potential as inducer of ISR where the interaction has been mentioned to interfere with the auto regulation of nodulation in alfalfa (Solans *et al.*, 2009). *Streptomyces* successfully suppresses the growth of plant pathogenic fungi (Riedlinger *et al.*, 2006). This is not to say that all *Streptomyces* spp. are PGPR. The plant growth promoter as well as plant growth retardant constitutes the genus *Actinomycetes*.

Conclusions and future perspectives :

The soil contains numerous genera of bacteria, many of which not only have important roles in nutrient cycling but also protect crops against diseases. Plant growth

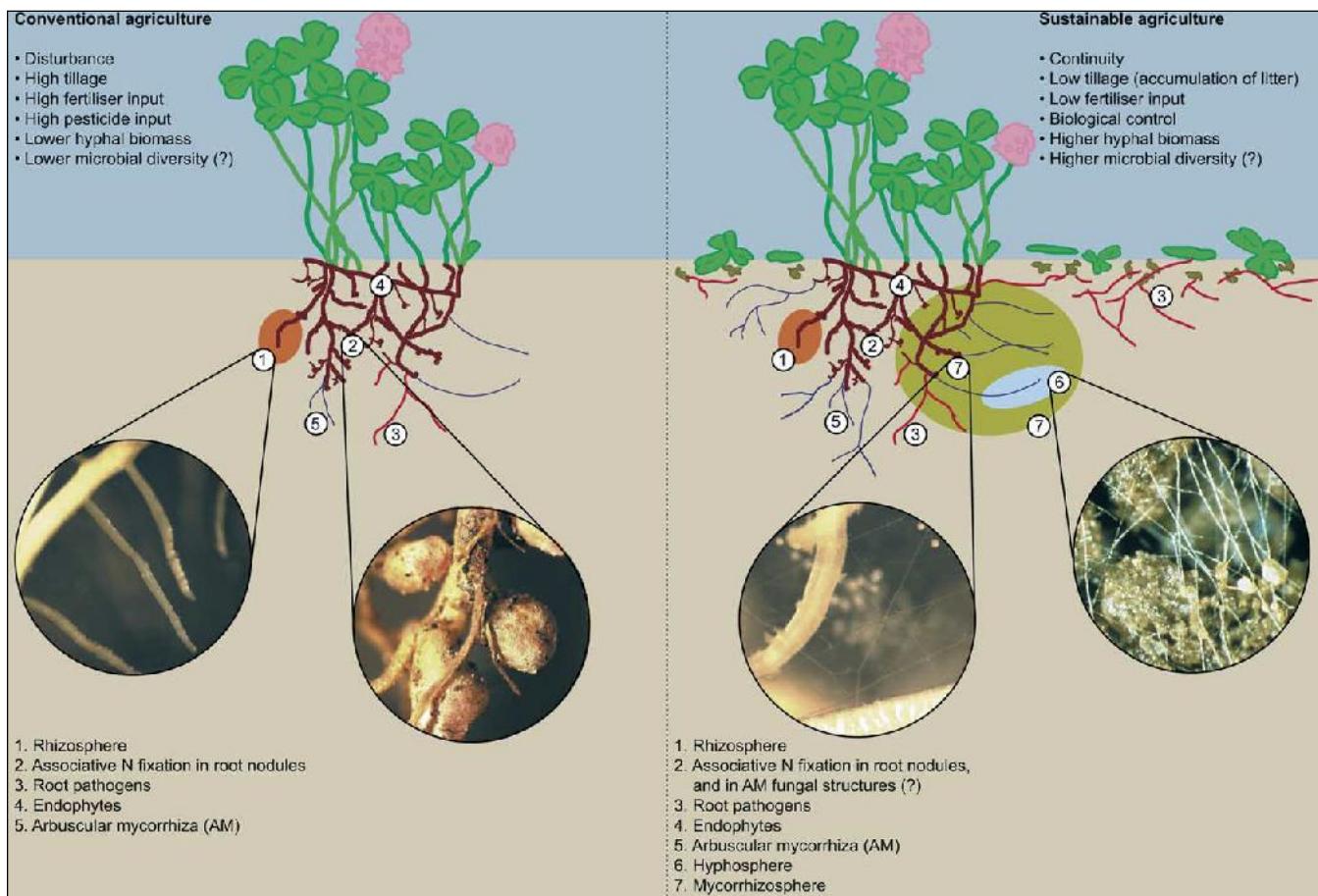


Fig. 1 : Schematic view of the Mycorrhizosphere concept in contrast to the Rhizosphere concept: Features of conventionally managed agricultural soils versus sustainably managed agricultural soils are indicated with emphasis on mycorrhizosphere components and possible effects on them (Source:- Joans et al., 2004).

promoting *Rhizobacteria* (PGPR) benefit the growth and development of plants directly and indirectly through several mechanisms. Chemical fertilizers increase yield in agriculture but are expensive and harm the environment. They deplete non-renewable energy via side effects, such as leaching out, and polluting water basins, destroying micro-organisms and friendly insects, making the crop more susceptible to the attack of diseases, reducing soil fertility, thereby causing irreparable damage to the overall system. The use of PGPR could be a better alternative to chemical fertilizers. They are economical, not harmful to the environment and could easily be found.

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